

## Review Article

# Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity

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Received 9 May 2013; Accepted 16 July 2013

Academic Editor: Nikhat J. Siddiqi

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The rapidly emerging field of nanotechnology has offered innovative discoveries in the medical, industrial, and consumer sectors. The unique physicochemical and electrical properties of engineered nanoparticles (NP) make them highly desirable in a variety of applications. However, these novel properties of NP are fraught with concerns for environmental and occupational exposure. Changes in structural and physicochemical properties of NP can lead to changes in biological activities including ROS generation, one of the most frequently reported NP-associated toxicities. Oxidative stress induced by engineered NP is due to acellular factors such as particle surface, size, composition, and presence of metals, while cellular responses such as mitochondrial respiration, NP-cell interaction, and immune cell activation are responsible for ROS-mediated damage. NP-induced oxidative stress responses are torch bearers for further pathophysiological effects including genotoxicity, inflammation, and fibrosis as demonstrated by activation of associated cell signaling pathways. Since oxidative stress is a key determinant of NP-induced injury, it is necessary to characterize the ROS response resulting from NP. Through physicochemical characterization and understanding of the multiple signaling cascades activated by NP-induced ROS, a systemic toxicity screen with oxidative stress as a predictive model for NP-induced injury can be developed.

## 1. Introduction

The growing field of nanotechnology has transformed many sectors of the industrial field with their breakthrough applications in the areas of biotechnology, electronics, medicinal drug delivery, cosmetics, material science, aerospace engineering, and biosensors. Manufactured nanomaterials (NM) have gained commercial interest in a variety of consumer products. Their novel physicochemical, thermal, and electrical properties facilitate their application in clothing, medicine, and cosmetics thereby increasing the probability for human and environmental contact with these NM [1–3]. Of all the NM, carbon nanotubes (CNT) and metal-based nanoparticles (NP) have generated considerable commercial interest owing to their remarkable intrinsic properties such as high tensile strength and conductivity, which in turn meet the needs of the specific application for which these NP are designed [4, 5]. Their widespread use raises concerns of

their inadvertent exposure in humans and the consequent deleterious health effects [6]. As compared to the growing commercial interest of NM, modest research effort has been invested in evaluating the potential adverse effects of these engineered NM. The sheer multiplicity of the physicochemical parameters of NM such as size, shape, structure, and elemental constituents makes the investigation of their toxic effects complex and challenging [7]. Some of the paradigms for NP-mediated toxicity include oxidative stress, inflammation, genetic damage, and the inhibition of cell division and cell death [8–11]. Most work to date has suggested that ROS generation (which can be either protective or harmful during biological interactions) and consequent oxidative stress are frequently observed with NP toxicity [3, 9]. The physicochemical characterization of NP including particle size, surface charge, and chemical composition is a key indicator for the resulting ROS response and NP-induced injury since many of these NP intrinsic properties can catalyze the

ROS production [6]. NP-mediated ROS responses have been reported to orchestrate a series of pathological events such as genotoxicity, inflammation, fibrosis, and carcinogenesis. For instance, CNT-induced oxidative stress triggers cell signaling pathways resulting in increased expression of proinflammatory and fibrotic cytokines [12]. Some NP have been shown to activate inflammatory cells such as macrophages and neutrophils which can result in the increased production of ROS [13–15]. Other NP such as titanium dioxide ( $\text{TiO}_2$ ), zinc oxide ( $\text{ZnO}$ ), cerium oxide ( $\text{CeO}_2$ ), and silver NP have been shown to deposit on the cellular surface or inside the subcellular organelles and induce oxidative stress signaling cascades that eventually result in oxidative stress to the cell [16]. The mechanism for ROS generation is different for each NP and to date the exact underlying cellular mechanism for ROS generation is incompletely understood and remains to be elucidated. Most of the metal-based NP elicit free radical-mediated toxicity via Fenton-type reactions [4, 17], whereas mitochondrial damage plays a major role in CNT-mediated ROS generation [18]. However, it is inaccurate to assume that ROS generation is a prerequisite to NP-induced toxicity since a few studies have reported the direct toxicity of NP without causing ROS [19]. Nevertheless, ROS generation is a major event during NP-induced injury that needs to be thoroughly characterized in order to predict NP-induced toxicity. This review will focus on oxidative stress as a mechanism for understanding NP-induced toxicity. For this paper, we have considered metal-based NP and CNT in the light of oxidative stress. The relationship between different NP characteristics and resulting oxidative stress is discussed.

**1.1. Generation of ROS.** ROS, key signaling molecules during cell signaling and homeostasis, are reactive species of molecular oxygen. ROS constitute a pool of oxidative species including superoxide anion ( $\text{O}_2^{\bullet-}$ ), hydroxyl radical ( $\text{OH}^\bullet$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), singlet oxygen ( $^1\text{O}_2$ ), and hypochlorous acid ( $\text{HOCl}$ ). ROS are generated intrinsically or extrinsically within the cell. Molecular oxygen generates  $\text{O}_2^{\bullet-}$ , the primary ROS via one-electron reduction catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Further reduction of oxygen may either lead to  $\text{H}_2\text{O}_2$  or  $\text{OH}^\bullet$  via dismutation and metal-catalyzed Fenton reaction, respectively [20, 21]. Some of the endogenous sources of ROS include mitochondrial respiration, inflammatory response, microsomes, and peroxisomes, while engineered NM, environmental pollutants act as exogenous ROS inducers. Physiologically, ROS are produced in trace amounts in response to various stimuli. Free radicals occur as essential byproducts of mitochondrial respiration and transition metal ion-catalyzed Fenton-type reactions [20]. Inflammatory phagocytes such as neutrophils and macrophages induce oxidative outburst as a defense mechanism towards environmental pollutants, tumor cells, and microbes. A variety of NP including metal oxide particles induce ROS as one of the principal mechanisms of cytotoxicity [22]. NP have been reported to influence intracellular calcium concentrations, activate transcription factors, and modulate cytokine production via generation of free radicals [12, 23].

**1.2. Oxidative Stress.** Abundance of ROS can have potentially damaging biological responses resulting in oxidative stress phenomenon. It results from an imbalance between the production of ROS and a biological system's ability to readily detoxify the reactive intermediates or repair the resulting damage. To overcome the excess ROS response, cells can activate enzymatic and nonenzymatic antioxidant systems [24]. The hierarchical model of oxidative stress was proposed to illustrate a mechanism for NP-mediated oxidative stress [4, 9]. According to this model, cells and tissues respond to increasing levels of oxidative stress via antioxidant enzyme systems upon NP exposure. During conditions of mild oxidative stress, transcriptional activation of phase II antioxidant enzymes occurs via nuclear factor (erythroid-derived 2)-like 2 (Nrf2) induction. At an intermediate level, redox-sensitive mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain enhancer of activated B cells (NF- $\kappa$ B) cascades mount a proinflammatory response. However, extremely toxic levels of oxidative stress result in mitochondrial membrane damage and electron chain dysfunction leading to cell death. Some of the key factors favoring the prooxidant effects of engineered NM include either the depletion of antioxidants or the increased production of ROS. Perturbation of the normal redox state contributes to peroxide and free radical production that has adverse effects on cell components including proteins, lipids, and DNA [23]. Given its chemical reactivity, oxidative stress can amount to DNA damage, lipid peroxidation, and activation of signaling networks associated with loss of cell growth, fibrosis, and carcinogenesis [16, 25, 26]. Besides cellular damage, ROS can result from interactions of NP with several biological targets as an effect of cell respiration, metabolism, ischemia/reperfusion, inflammation, and metabolism of various NM [22]. Most significantly, the oxidative stresses resulting from occupational NM exposures as well as experimental challenge with various NP lead to airway inflammation and interstitial fibrosis [27–30].

**1.3. Nanoparticle-Induced Oxidative Stress.** Nanomaterials of varying chemical composition such as fullerenes, CNT, and metal oxides have been shown to induce oxidative stress [20, 31]. The key factors involved in NP-induced ROS include (i) prooxidant functional groups on the reactive surface of NP; (ii) active redox cycling on the surface of NP due to transition metal-based NP; and (iii) particle-cell interactions [22, 25]. From a mechanistic point of view, we discuss the sources of ROS based on the physicochemical parameters and particle-cell interactions.

Several studies demonstrate the significance of reactive particle surface in ROS generation [20, 32]. Free radicals are generated from the surface of NP when both the oxidants and free radicals bound to the particle surface. Surface bound radicals such as  $\text{SiO}^\bullet$  and  $\text{SiO}_2^\bullet$  present on quartz particles are responsible for the formation of ROS such as  $\text{OH}^\bullet$  and  $\text{O}_2^{\bullet-}$  [17, 25]. Ambient matter such as ozone and nitrogen dioxide ( $\text{NO}_2$ ) adsorbed on the particle surface is capable of inducing oxidative damage [16]. Reduced particle size results in structural defects and altered electronic properties

on the particle surface creating reactive groups on the NP surface [27, 33]. Within these reactive sites, the electron donor or acceptor active sites interact with molecular  $O_2$  to form  $O_2^{\bullet-}$  which in turn can generate additional ROS via Fenton-type reactions [3]. For instance, NP such as Si and Zn with identical particle size and shape lead to diverse cytotoxicity responses due to their surface properties. ZnO being more chemically active than  $SiO_2$ , led to increased  $O_2^{\bullet-}$  formation resulting in oxidative stress. Free radicals are either directly bound to the NP surface or may be generated as free entities in an aqueous suspension [17]. Dissolution of NP and subsequent release of metal ions can enhance the ROS response [25]. For instance, aqueous suspensions of quartz particles generate  $H_2O_2$ ,  $OH^{\bullet}$ , and  $^1O_2$  [17, 20, 32].

Apart from surface-dependent properties, metals and chemical compounds on the NP surface accelerate the ROS response [34]. Transition metals including iron (Fe), copper (Cu), chromium (Cr), vanadium (V), and silica (Si) are involved in ROS generation via mechanisms such as Haber-Weiss and Fenton-type reactions [25]. Fenton reactions usually involve a transition metal ion that reacts with  $H_2O_2$  to yield  $OH^{\bullet}$  and an oxidized metal ion. For example, the reduction of  $H_2O_2$  with ferrous iron ( $Fe^{2+}$ ) results in the formation of  $OH^{\bullet}$  that is extremely reactive and toxic to biological molecules [21]. Cu and Fe metal NP have been reported to induce oxidative stress ( $O_2^{\bullet-}$  and  $OH^{\bullet}$ ) via Fenton-type reaction [26], while the Haber-Weiss-type reaction involves a reaction between oxidized metal ion and  $H_2O_2$  to induce  $OH^{\bullet}$  [21, 35]. NP including chromium, cobalt, and vanadium can catalyze both Fenton and Haber-Weiss-type reactions [26]. Glutathione reductase, an antioxidant enzyme, reduces metal NP into intermediates that potentiate the ROS response. In addition, some metal NP (Ar, Be, Co, and Ni) promote the activation of intercellular radical-inducing system such as the MAPK and NF- $\kappa$ B pathways [36].

In addition to the prooxidant effect of NP, ROS are also induced endogenously where the mitochondrion is a major cell target for NP-induced oxidative stress. Once NP gain access into the mitochondria, they stimulate ROS via impaired electron transport chain, structural damage, activation of NADPH-like enzyme system, and depolarization of the mitochondrial membrane [37, 38]. For instance, cationic polystyrene nanospheres induce  $O_2^{\bullet-}$  mediated apoptosis in murine macrophages based on their ability to target mitochondria [38].

Cellular internalization of NP has been shown to activate immune cells including macrophages and neutrophils, contributing to ROS/RNS [22, 25]. This process usually involves the activation of NADPH oxidase enzymes. *In vivo* particle exposures such as silica activate the rich pool of inflammatory phagocytes within the lung causing them to induce oxidative outburst [39]. NP with smaller particle size are reported to induce higher ROS owing to their unique characteristics such as high surface to volume ratio and high surface charge. Particle size determines the number of reactive groups/sites on the NP surface [34, 37, 40]. The pulmonary responses induced by inhaled NP are considered to be greater than those produced by micron-sized particles because of the increased surface area to particle mass ratio [28]. Larger surface area

ensures that the majority of the molecules are exposed to the surface than the interior of the NM [3]. Accordingly, nano-sized  $SiO_2$  and  $TiO_2$  and MWCNT induce greater ROS as compared to their larger counterparts [41]. Additionally, a study with cobalt/chromium NP exposure demonstrated particle size dependent ROS-mediated genotoxicity [42].

## 2. Oxidant Generation via Particle-Cell Interactions

Besides being self-oxidative in nature, NP react with cells and induce their prooxidant effects via intracellular ROS generation involving mitochondrial respiration and activation of NADPH-like enzyme systems [43]. NP can activate the cellular redox system specifically in the lungs where immune cells including alveolar macrophages (AM) and neutrophils act as direct ROS inducers. Professional phagocytic cells including neutrophils and AM of the immune system induce substantial ROS upon internalization of NP via the NADPH oxidase enzyme system [44]. The phagocytic oxidative outburst is attributable to some of the NP physicochemical properties. In case of silica and quartz particles, inflammation-induced ROS was associated with the surface-based radical-generating properties of the particles [45]. Additionally, NP from the residual oily fly ash and diesel exhaust activate the pool of inflammatory phagocytes resulting in massive ROS release [46]. Furthermore, adsorption of chemicals such as organic matter onto the NP surface may drive the inflammation-induced oxidative stress [24].

**2.1. Lung Injury Caused by Nanoparticle-Induced Reactive Nitrogen Species.** Besides oxidative damage, NP exposure within the lung is reported to induce reactive nitrogen species (RNS). Particle deposition in the lung causes recruitment of inflammatory cells that generate ROS, clastogenic factors, and cytokines either harming or stimulating resident lung cells [31]. Inflammatory phagocytes are an important source of RNS/ROS generation within the lung. Owing to their inducible nitric oxide synthase (iNOS) activity, phagocytes can produce a large amount of genotoxic RNS, including nitric oxide ( $NO^{\bullet}$ ) and the highly reactive peroxynitrite ( $ONOO^-$ ).  $ONOO^-$  formed by the reaction of  $NO^{\bullet}$  and  $O_2^{\bullet-}$  causes DNA fragmentation, lipid oxidation, and protein dysfunction consequently contributing to particle-induced lung injury [47]. *In vivo* exposure to  $SiO_2$  and quartz NP elicited an RNS response characterized by increased iNOS and  $NO^{\bullet}$  within the lung as a result of phagocyte influx [48, 49].

**2.2. Mechanisms of ROS Production and Apoptosis within Metal Nanoparticles.** Apoptosis has been implicated as a major mechanism of cell death caused by NP-induced oxidative stress [50–52]. Among the different apoptotic pathways, the intrinsic mitochondrial apoptotic pathway plays a major role in metal oxide NP-induced cell death since mitochondria are one of the major target organelles for NP-induced oxidative stress [38]. High levels of ROS in the mitochondria can



result in damage to membrane phospholipids inducing mitochondrial membrane depolarization [53]. Small proportion of electrons escapes the mitochondrial chain and interacts with molecular oxygen to form  $O_2^{\bullet-}$  which later gives rise to  $H_2O_2$  or partially reduces to the damaging  $OH^\bullet$ . NP can catalyze the  $O_2^{\bullet-}$  generation either by blocking the electron transport chain or accelerating electron transfer to molecular oxygen [54, 55]. Various metal oxide NP including Zn, Cu, Ti, and Si elicit ROS-mediated cell death via mitochondrial dysfunction [56–59].

### 3. Introduction to Transition Metals

Transition metal oxide particles have been used to revolutionize several fields including catalysis, sensors, optoelectronic materials, drug delivery, automobile, and material science engineering. Apart from industrial scale applications, metal NP are increasingly used in a variety of consumer products such as cosmetics, sunscreens, textiles, and food products. Among the transition metal oxides, titanium dioxide, cupric oxide, and zinc oxide have gained attention owing to their commercial usage [60]. Metal oxide particles can undergo surface modification for better stability and binding to other substrates. Such widespread applications are attributable to their electrochemical and physical properties reflecting their small sizes and reactive surfaces. For example, a relatively inert metal or metal oxide may become a highly effective catalyst when manufactured as NP. Their fixed particle mass, high aspect ratio, and particle surface bioreactivity tailor them to meet the needs of specific application. However, a high surface-to-volume ratio makes NP reactive and exposes them to environmental stressors, particularly free radical generation [61, 62]. Besides, the nanoscale dimensions enhance their cellular uptake and interaction with biological tissues. Metals can generate free radicals via the Fenton-type reactions that react with cellular macromolecules and induce oxidative stress [63]. The toxicity of metallic NP including Zn, Ti, Si, Fe, and Ce has been characterized by increased ROS generation and oxidative stress and apoptosis [61, 64–66]. The oxidative stress mediated outcomes of various metal NP are summarized in Table 1.

### 4. Prooxidant Effects of Metal Oxide Nanoparticles

To overcome the overwhelming ROS production, cells trigger either a defensive or an injurious response eliciting a chain of adverse biological responses. Free radicals are potentially damaging to cellular macromolecules including lipids, proteins, and nucleic acids. DNA is one of the major targets for oxidative stress and represents the first step involved in mutagenesis, carcinogenesis, and aging. ROS/RNS cause oxidative DNA damage in the form of DNA strand breaks, DNA protein cross-links, and alkali-labile sites [67, 68], and given their characteristic nature free radicals appear as one of the likely carcinogens [25, 69]. Testing the genotoxic potential is essential for carcinogenic risk assessment of NP. Genotoxic effects may be produced either by direct interaction of

particles with genetic material or by secondary damage from particle-induced ROS. Transition metal NP induce chromosomal aberrations, DNA strand breaks, oxidative DNA damage, and mutations [70].  $OH^\bullet$ , one of the highly potent radicals, is known to react with all components of DNA causing DNA single strand breakage via formation of 8-hydroxyl-2'-deoxyguanosine (8-OHdG) DNA adduct [71, 72]. 8-OHdG is a biomarker of  $OH^\bullet$ -mediated DNA lesions. NP exposure significantly elevated 8-OHdG levels both *in vivo* [73] and *in vitro* [74], demonstrating their mutagenic behavior. A recent study comparing metal oxide NP including Cu, Fe, Ti, and Ag reported ROS-mediated genotoxicity characterized by micronuclei and DNA damage *in vivo* [75].

Along with chromosomal damage, free radicals also interact with lipids and proteins, abundantly present in biomembranes, to yield lipid peroxidation products associated with mutagenesis. Polyunsaturated fatty acids are subject to oxidation giving rise to lipid hydroperoxides as the initial step in ROS generation [25, 76]. Prooxidant metals such as Cu and Fe react with these lipid hydroperoxides to induce DNA damaging end-products malondialdehyde (MDA) and 4-hydroxynonenal that act as inflammatory mediators and risk factors for carcinogenesis. Exposures to metal oxide NP of Ti, Cu, Si, and Fe were reported to induce tissue damage, abnormal cellular stress response via lipid peroxidation [77–79].

Alterations within the antioxidant defense system pose as a risk factor for carcinogenesis [68]. Glutathione (GSH) a potent free-radical scavenger, is responsible for maintaining the cellular redox state and protecting cells from oxidative damage [80, 81]. NP-triggered free radicals reduce GSH into its oxidized form glutathione disulfide (GSSG), thereby contributing to oxidative stress, apoptosis, and sensitization to oxidizing stimuli [82, 83]. Apart from GSH, NP-induced ROS modulate the antioxidant activities of ROS-metabolizing enzymes including NADPH-dependent flavoenzyme, catalase, glutathione peroxidase, and superoxide dismutase [84].

It is well established that uncontrolled generation of ROS triggers a cascade of proinflammatory cytokines and mediators via activation of redox sensitive MAPK and NF- $\kappa$ B signaling pathways that control transcription of inflammatory genes such as IL-1 $\beta$ , IL-8, and TNF- $\alpha$  [21]. Oxidative stress plays a key role in NP-induced airway hypersensitivity and respiratory inflammation [85]. A study involving coexposure of metal oxide NP with a bacterial endotoxin demonstrated exaggerated lung inflammation and pulmonary edema [86]. Additionally, studies with different metal oxide NP have demonstrated ROS-mediated inflammatory response. For instance, SiO<sub>2</sub> and TiO<sub>2</sub> NP induce an elevated inflammatory response through the underlying mechanism of ROS generation [64, 85, 87]. Pulmonary inflammation may induce changes in membrane permeability, facilitating NP distribution beyond the lung and indirectly affecting cardiovascular performance [88].

Metal ion-induced free radicals can activate oncogenes such as Ras [25]. Excess amounts of NP have been associated with skin, bladder, liver, lung, and respiratory tract cancers [7]. Transition metals in trace amounts are introduced during the manufacture and preparation of CNT. Given

TABLE 1: List of studies describing the ROS-dependent effects of metal-based NP.

Nanoparticles	ROS-dependent effect	Reference
Iron oxide		
Iron oxide	Necrosis and apoptosis in murine macrophage (J774) cells	[61]
Zero-valent iron	Acute cytotoxicity in human bronchial epithelial cells	[140]
Iron oxide	Human microvascular endothelial cell permeability	[141]
SPIONS	Activation of NF- $\kappa$ B and AP-1, inflammation in human epidermal keratinocytes (HEK) and murine epidermal cells (JB6 P(+))	[142]
Copper oxide		
Copper oxide	Genotoxicity in human lung epithelial cells	[143]
Copper oxide	Mitochondrial dysfunction, oxidative DNA damage, cell death in A549 cell line	[144]
Copper oxide	Cytotoxicity <i>in vitro</i> in Hep-2 cell line	[145]
Copper oxide	Nephrotoxicity and hepatotoxicity <i>in vivo</i>	[146]
Cerium oxide		
Cerium oxide	Lung inflammation and alveolar macrophage apoptosis <i>in vivo</i>	[147]
Cerium oxide	Apoptosis via caspase-3 activation and chromatin condensation <i>in vitro</i> in BEAS-2B cells	[64]
Cerium oxide	HO-1 induction via the p38-Nrf-2 signaling pathway <i>in vitro</i> in BEAS-2B cell line	[148]
Cerium oxide	Lipid peroxidation and membrane damage <i>in vitro</i> in lung cancer cells	[149]
Zinc oxide		
Zinc oxide	Mitochondrial dysfunction, morphological modification, and apoptosis <i>in vitro</i> in human fetal lung fibroblasts	[59]
Zinc oxide	Cellular oxidant injury, excitation of inflammation, and cell death in BEAS-2B and RAW 264.7 cells	[150]
Zinc oxide	Mitochondrial damage, apoptosis, and IL-8 release <i>in vitro</i> in LoVo human colon carcinoma cell line	[151]
Zinc oxide	Mitochondrial damage, genotoxic and apoptotic cell effects <i>in vitro</i> human liver cells	[152]
Zinc oxide	Genotoxic and apoptotic responses <i>in vitro</i> in human skin melanoma cell line (A375)	[153]
Zinc oxide	Endoplasmic reticulum stress, apoptosis, and necrosis in rat retinal ganglion cells	[154]
Zinc oxide nanorods	Apoptosis in human alveolar adenocarcinoma cells via p53, surviving, and bax/bcl-2 pathways	[155]
Nanosilica		
Nanosilica	Cytotoxicity and apoptosis via activation of p53 and Bax <i>in vitro</i> in human hepatic cell line	[156]
Nanosilica	p53 and p21 mediated G1 phase arrest <i>in vitro</i> myocardial H9c2 (2-1) cells	[157]
Nanosilica	Cell cycle arrest and apoptosis <i>in vitro</i> human embryonic kidney cell line	[158]
Nanosilica	Hepatotoxicity <i>in vitro</i> in Kupffer cells and ROS-mediated cell death, oxidative DNA damage	[159]
Nickel oxide		
Nickel oxide	Lipid peroxidation, apoptosis <i>in vivo</i> in human epithelial airway cells	[160]
Nickel ferrite	Apoptosis in A549 cells through oxidative stress via p53, survivin, bax/bcl-2, and caspase pathways in normal Chang (normal human liver), MCF10A (normal breast epithelial), and WI38 (normal lung fibroblast) cell lines	[161]
Titanium dioxide		
Titanium dioxide	Apoptotic cell death through ROS-mediated Fas upregulation and Bax activation	[162]
Titanium dioxide	Cytotoxic and genotoxic effects <i>in vitro</i> in human amnion epithelial (WISH) cell line	[163]
Titanium dioxide	Cytotoxicity and apoptotic cell death <i>in vitro</i> in HeLa cell line	[164]
Aluminum oxide		
Aluminium oxide	Mitochondria mediated oxidative stress and cytotoxicity in human mesenchymal stem cells	[165]
Gold		
Gold	Lipid peroxidation and autophagy <i>in vitro</i> in MRC-5 lung fibroblasts	[166]
Silver		
Ag-NP	Mitochondrial damage and genotoxicity in human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251)	[167]
Ag-NP	JNK-mediated mitochondrial apoptosis in NIH3T3 fibroblasts	[50]
Ag-NP	Mitochondrial damage, apoptosis <i>in vitro</i> in A549 cells	[168]
Cobalt-chromium (Co-Cr)		
Co-Cr NP	Oxidative DNA damage, micronuclei induction, reduced cell viability in human dermal fibroblasts	[169]

their oxidizable nature, studies suggest that metals including Fe, Co, and Ni are more toxic and fibrogenic upon their interaction with CNT as compared to pure CNT [89–93]. Vanadium pentoxide ( $V_2O_5$ ), a transition metal byproduct of petrochemicals, is associated with fibrosis via generation of  $H_2O_2$  and other ROS [94]. Occupational exposures to combustion-derived NP such as welding fumes consisting of metals such as Fe, Mn, Si, Cr, and Ni induce fibrogenic responses [95]. Metal containing welding fume NP elicited ROS-dependent lipid peroxidation and inflammation *in vivo* [96, 97].

## 5. Cellular Signaling Affected by Metal Nanoparticles

The prooxidant effects of NP result in the activation of signaling pathways, transcription factors, and cytokine cascade contributing to a diverse range of cellular responses. The regulation of redox homeostasis entails signaling cascades such as HIF-1, NF- $\kappa$ B, PI3 K, and MAPK which control proliferation, metastasis, cell growth, apoptosis, survival, and inflammation [7, 12]. At an intermediate level of oxidative stress, proinflammatory pathways are activated in an attempt to maintain the redox equilibrium. The inflammatory cascade involves profibrotic mediators such as TNF- $\alpha$ , IL-1 $\beta$ , and TGF- $\beta$  which have been implicated in the pathogenesis of fibrosis. Cells are known to counteract the overwhelming oxidative stress response via increased cytokine expression such as interleukins and TNF- $\alpha$ , activation of kinases, and inhibition of phosphatases thereby influencing the phosphorylation cascade. Protein phosphorylation is involved in the regulation of critical cellular responses including mitogenesis, cell adhesion, oncogenic transformation, and apoptosis. Thus, ROS response appears to be closely related to factors driving carcinogenesis [98].

**5.1. NF- $\kappa$ B.** The NF- $\kappa$ B group of proteins activates genes responsible for defense mechanisms against cellular stress and regulates miscellaneous functions such as inflammation, immune response, apoptosis, and cell proliferation. Prooxidant  $H_2O_2$ -mediated NF- $\kappa$ B activation through the classical IKK-dependent pathway is well established. ROS such as  $OH^\bullet$ ,  $HOCl$ , and  $^1O_2$  and RNS such as  $ONOO^-$  activate NF- $\kappa$ B via the release of I $\kappa$ Bs resulting in the nuclear translocation of NF- $\kappa$ B [99, 100]. Once inside the nucleus, NF- $\kappa$ B induces transcription of proinflammatory mediators resulting in inflammation and oxidative stress. During NP-mediated lung injury, ROS activate NF- $\kappa$ B to modulate the production of proinflammatory TNF- $\alpha$ , IL-8, IL-2, and IL-6 from macrophages and lung epithelial cells [101]. Several metal oxide NP such as Zn, Cd, Si, and Fe exert their toxic effects via ROS-dependent NF- $\kappa$ B activation [62, 102, 103].

**5.2. AP-1.** Activator protein (AP)-1 is a transcription factor activated in response to oxidants, cytokines, growth factors, and bacterial and viral infections. It is responsible for regulation of cell proliferation, differentiation, and apoptosis, thereby it is a key factor in carcinogenesis [104]. Activation

of AP-1 under oxidative conditions is believed to be mediated via phosphorylation of protooncogene c-jun [68]. Metal NP including Cr, Ni, and Fe have been shown to activate AP-1 via ROS generation [60].

**5.3. MAPK.** MAPK are serine-threonine protein kinases that control a diverse range of cellular responses including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis. MAPK consist of growth factor-regulated extracellular signal-related kinases (ERK) and the stress-activated MAPK, c-jun NH<sub>2</sub>-terminal kinases (JNK), and p38 MAPK. Once ROS production exceeds the capacity of the antioxidant proteins, free radicals may induce oxidative modification of MAPK signaling proteins (e.g., RTK and MAP3 K), thereby leading to MAPK activation. ROS may activate MAPK pathways via inhibition and/or degradation of MAPK phosphatases (MKP) [105, 106]. Finally, the site of ROS production and the concentration and kinetics of ROS production as well as cellular antioxidant pools and redox state are most likely to be important factors in determining the effects of ROS on activation of MAPK pathways [107]. Ag-NP activate JNK protein signaling and apoptosis in a variety of cells [50], whereas  $CeO_2$  NP trigger p38 MAPK signaling in bronchoalveolar cells [64].

**5.4. PTP.** Protein tyrosine phosphatases (PTP) are key regulatory components in signal transduction pathways involved in cell growth, differentiation, proliferation, and transformation. The highly reactive cysteine residues of PTP are predisposed to oxidative stress in the form of  $H_2O_2$ , free radicals or changes in intracellular thiol/disulfide redox state [98, 108]. Metal NP including  $Zn^{2+}$  and  $V^{5+}$  may be critical in redox regulation of PTP via the inhibition of MAPK and EGFR [109, 110].

**5.5. Src.** Src kinases belong to the nonreceptor tyrosine kinase family involved in the regulation of cell growth. Mild oxidative stress is sufficient to activate Src kinase which later triggers a cell signaling cascade [111]. This may explain the low dose of metal NP-induced lymphocyte cell death via ROS-dependent activation of Src kinases [112].

## 6. Carbon Nanotubes

One of the most promising materials in the field of nanotechnology is CNT, and their widespread applications are attributable to the diverse physical, chemical, and electrical characteristics they possess. CNT are high aspect ratio nanomaterials (HARN) having at least one of their dimensions in the order of 100 nm or less according to the British Standards Institute Report [113]. CNT are made of either single-walled (SW) or multiwalled (MW) graphite layers. With unique properties such as high tensile strength and conductivity, they have been explored in the areas of electronics, biotechnology, medicinal drug delivery, cosmetics, material science, and aerospace engineering. CNT structure facilitates their entry, deposition, and residence in the lungs and pleura, resulting in incomplete phagocytosis and clearance from the lungs [5].

Owing to their biopersistent and nonbiodegradable nature, and particularly their resemblance to needle-like asbestos fibers, CNT are believed to induce biologically harmful effects [89]. Physicochemical parameters such as particle size, surface modification, presence of metals, surface reactivity, and surface charge are responsible for the prooxidant effects of CNT. Frustrated phagocytosis of CNT has been implied in CNT-induced oxidative stress.

## 7. Carbon Nanotube-Induced Oxidative Stress

One of the most frequently reported toxicity endpoints for CNT is the formation of ROS which can be either protective or harmful during biological interactions. Oxidative stress may be caused directly by CNT-induced ROS in the vicinity or inside the cell or could arise more indirectly due to the effects of internalized CNT on mitochondrial respiration [114] or in depletion of antioxidant species within the cell [64]. Moreover, NADPH-mediated ROS are critical for SWCNT-induced pulmonary responses [91]. The most likely mechanism for CNT-induced oxidative stress and lung toxicity involves mitochondrial dysfunction. Incomplete phagocytosis of CNT, presence of transition metals and specific reactive groups on the CNT surface are key drivers of ROS generation. Metal impurities such as Fe, Co, and Ni introduced within the CNT during their synthesis are key factors driving CNT-mediated ROS response [115, 116]. CNT-induced oxidative stress mediates important cellular processes including inflammation, cell injury, apoptosis, and activation of cellular signaling pathways such as MAPK and NF- $\kappa$ B which are implicated in the pathogenesis of lung fibrosis [31, 117]. For instance, SWCNT dependent OH<sup>•</sup> generation leads to activation of molecular pathways MAPK, AP-1, NF- $\kappa$ B, and Akt associated with cell proliferation and tumor progression *in vitro* [93]. Several studies demonstrate SWCNT-induced oxidative stress [118–120]. Similarly, MWCNT exposures have been reported to induce ROS both *in vitro* and *in vivo* [18, 121–123]. Interestingly, oxidative stress is reported to be a mechanism for biodegradation of CNT. SWCNT undergoes oxidative biodegradation via myeloperoxidase, a prooxidant enzyme involved in host defense responses [120]. Table 2 summarizes the different studies that report ROS-dependent effects of CNT.

## 8. Role of ROS in CNT-Induced Inflammation

ROS and inflammation demonstrate an interdependent relationship in the case of exposure to NP. Inflammatory cells such as macrophages and neutrophils induce enormous ROS release in order to get rid of the NP. However, NP exposure-mediated oxidative stress leads to activation of RTK, MAPK, Akt, and NF- $\kappa$ B contributing to the proinflammatory cascade [124]. Accordingly, CNT-induced ROS were reported to elicit pro-inflammatory transcription factors such as NF- $\kappa$ B, AP-1 and MAPK *in vivo*. This was found to be an inflammation dependent response [93]. MWCNT treatment in macrophages mediates ROS-dependent activation of NF- $\kappa$ B pathway, thereby inducing the expression of chemokines

and cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and MCP-1 [18]. Likewise, MWCNT-induced nitrosative stress *in vivo* is associated with pulmonary inflammation [125].

## 9. Role of ROS in CNT-Induced Genotoxicity

CNT elicit genotoxic effects through direct interaction with DNA or indirectly via CNT-induced oxidative stress and inflammatory responses. CNT-induced sustained oxidative stress can result in DNA damage and abnormal cell growth, possibly leading to carcinogenesis and fibrogenesis [126, 127]. A plethora of studies demonstrate the genotoxic potential of both MWCNT and SWCNT [128–131]. ROS can activate cellular signaling pathways resulting in cell cycle arrest and apoptosis. CNT induce a multitude of genotoxic responses including DNA strand breakage, oxidation, micronuclei induction, chromosomal aberrations, formation of  $\gamma$ H2AX foci, and mutant frequencies [132]. Oxidative stress-dependent DNA breakage and repair and activation of signaling pathways including poly-ADP-ribose polymerase (PARP), AP-1, NF- $\kappa$ B, p38, and Akt were reported in human mesothelial cells exposed to SWCNT [93]. CNT induce ROS-dependent lipid peroxidation both *in vitro* and *in vivo* [133, 134]. A number of studies account for mitochondrial membrane depolarization, damage, and oxidative stress upon CNT exposure [92, 135, 136]. Unlike the traditional prooxidant effect of NP, CNT have been reported to sequester ROS which in turn is associated with their structural defects [83]. This quenching is reported to be related to the genotoxic and inflammatory effects observed with CNT [137].

## 10. Role of ROS in CNT-Induced Fibrosis

Increased ROS has been implicated in lung inflammation and fibrosis. The inflammatory cascade is reported to contribute to oxidative stress mediated lung injury [138]. Exposure to CNT results in expression of genes responsible for inflammation and fibrosis via the activation of cell signaling pathways and transcription factors including NF- $\kappa$ B, STAT-1, MAPK, and RTK [31]. ROS-dependent p38-MAPK has been shown to be responsible for CNT-induced collagen and angiogenic responses [118]. Additionally, SWCNT induce fibrogenic effects via ROS-mediated NF- $\kappa$ B activation [139], whereas MWCNT induce fibroblast to myofibroblast differentiation via ROS-dependent NF- $\kappa$ B activation [18].

## 11. Oxidative Stress as an Underlying Mechanism for NP Toxicity

Findings from several studies have pointed out that ROS generation and oxidative stress occur as an early event leading to NP-induced injury. Oxidative stress corresponds with the physicochemical reactivity of NP including metal-based particles as well as the fibrous CNT. Oxidative stress related to NP exposure involves mitochondrial respiration, mitochondrial apoptosis, activation of NADPH oxidase system, alteration of calcium homeostasis, and depletion of antioxidant enzymes; all of which are associated with tissue injury. NP-driven ROS



TABLE 2: List of studies describing the ROS-dependent effects of CNT.

	CNT	
SWCNT with 30% iron by mass	Lipid peroxidation, reduced cell viability, and antioxidant reserve in human keratinocytes	[170]
Acid treated MWCNTs with Co and Ni	Decreased cell viability, altered mitochondrial membrane potential in rat macrophages (NR8383) and human A549 lung cells	[92]
SWCNT	Reduced cell viability and antioxidant reserve in rat lung epithelial cells	[171]
SWCNT	Increased apoptosis, DNA damage, activated MAPKs, AP-1, NF- $\kappa$ B, and Akt in normal and malignant human mesothelial cells	[93]
SWCNT	Reduced cell proliferation, activation of NF- $\kappa$ B in human keratinocytes	[119]
Unpurified SWCNT (30% w/w iron)	Activation of AP-1 and NF- $\kappa$ B, cytotoxicity, and proinflammatory response <i>in vitro</i> and <i>in vivo</i>	[172]
Unpurified SWCNT (17.7% w/w iron)	Lipid peroxidation, acute inflammatory response, decreased respiratory function in adult C57BL/6 mice	[91]
Raw MWCNT	Dose-dependent cytotoxicity in RAW 264.7 macrophages and A549 cells: cell inflammation, membrane leakage, lipid peroxidation, and protein release	[173]
MWCNT	Increase in cell permeability, cell migration, and endothelial permeability in human microvascular endothelial cells (HMVEC)	[174]
SWCNT	Activation of p38 MAPK in CNT mediated fibrogenic and angiogenic responses <i>in vitro</i> in human lung fibroblasts	[118]
MWCNT	Activation of NF- $\kappa$ B, fibroblast-myofibroblast transformation, profibrogenic cytokine, and growth factor induction <i>in vitro</i> (BEAS-2B, WI-38, and A549 cell lines)	[18]

response contributes to activation of cell signaling pathways, inflammatory cytokine and chemokine expressions, and specific transcription factor activation. Activation of these cellular mechanisms is closely associated with transcription of genes involved in inflammation, genotoxicity, fibrosis, and cancer. Thus, the pathological consequences observed during NP exposure could be attributable to ROS generation. It is essential to incorporate these adverse biological responses as a screening tool for toxic effects of NP. For instance, over-expression of antioxidant enzymes is indicative of the mild oxidative stress, whereas mitochondrial apoptosis occurs during conditions of toxic oxidative stress. The hierarchical model of ROS response provides a scale to gauge the adverse health effects upon NP exposures. A NP exposure study must collectively involve rigorous characterization of NP and assign *in vitro* and *in vivo* oxidative stress markers as toxicity endpoints as a predictive paradigm for risk assessment [6, 9, 12]. Figure 1 summarizes the key findings regarding the oxidative effects of NP and resulting toxicity.

## 12. Conclusion

This paper reviews the cellular mechanisms of NP-induced oxidative stress and toxicity. We focus on the toxicity of metal oxide NP and CNT with respect to the oxidative stress paradigm. The principal factors for NP-induced oxidative stress involve (a) the oxidative properties of the NP themselves and (b) oxidant generation upon interaction of NP with cellular material. The direct prooxidant effects of NP are attributable to their physicochemical properties including surface reactivity, particle size, surface charge,

chemical composition, and the presence of transition metals. Therefore, it is necessary to ensure extensive characterization of the physicochemical properties for safer design and manufacture of NP. Whereas, ROS mediated via NP-cell interaction involve mechanisms including immune cell activation, mitochondrial respiration, and NADPH oxidase system. Apart from ROS, NP also arbitrate RNS-mediated injury. Given their chemical reactivity, metal-based NP induce oxidative damage to cellular macromolecules such as proteins, lipids, and DNA via Fenton-type and Haber Weiss-type reactions. The key pathophysiological outcomes of oxidative insults during metal NP exposures involve cell membrane damage, lipid peroxidation, protein denaturation, and alteration of calcium homeostasis. Furthermore, the findings in the review article suggest that CNT-induced oxidative stress is indicative of the pulmonary toxicity of CNT. Metal-based NP and fibrous CNT-mediated ROS result in activation of cell signaling pathways, transcription factor activation, cytokine mediator release, and apoptosis. The persistent activation of these signaling cascades has some clinical ramifications. Redox imbalance via engineered NP exerts undesirable pathophysiological outcomes such as genotoxicity, inflammation, fibrosis, and carcinogenesis. It is of utmost importance to understand the molecular and cellular mechanisms of NP-induced oxidative stress which in turn will yield novel strategies to mitigate the toxicity of engineered NP. Moreover, it necessitates the establishment of stringent procedures for testing the oxidative potential of manufactured NP prior to their commercialization. Identifying the major cellular targets for NP-induced ROS will facilitate safer design and manufacture of NM in the market place.



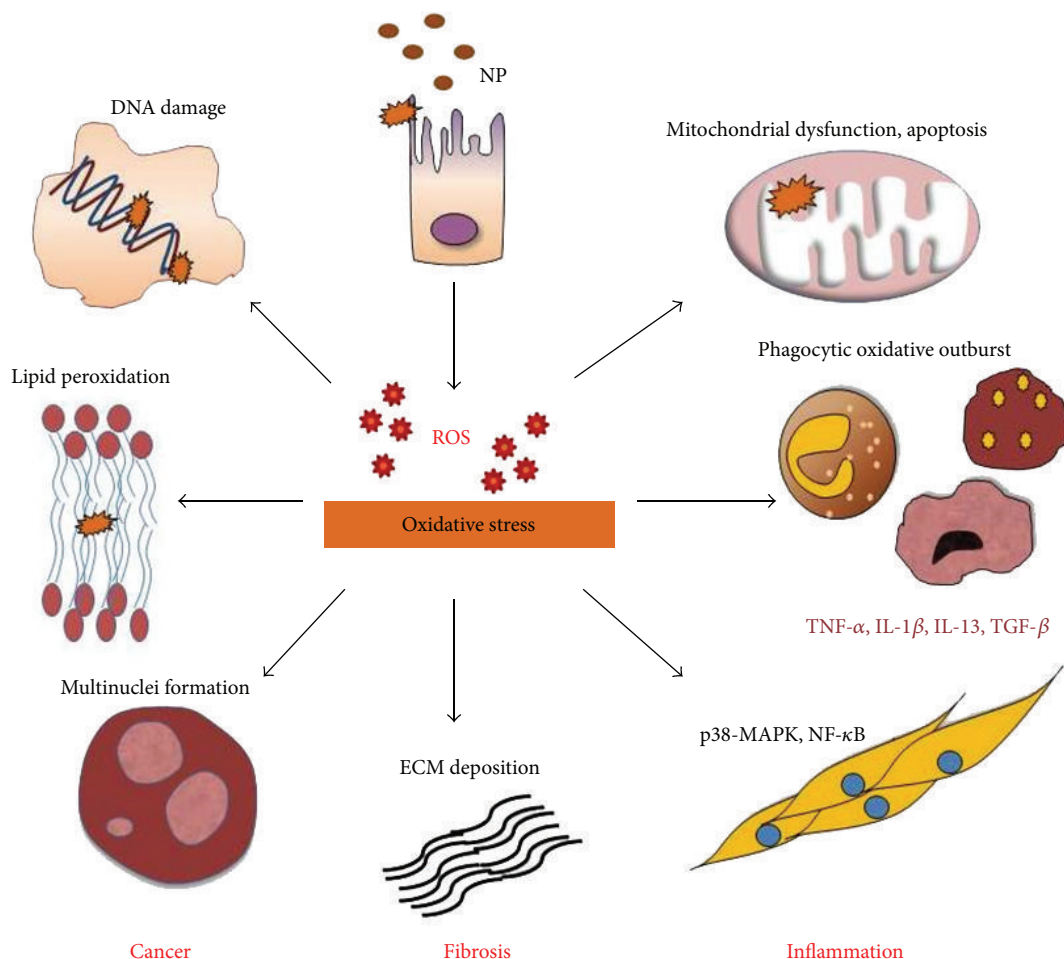


FIGURE 1: Prooxidant pathway for NP-induced toxicity: various NP exhibit oxidative stress dependent toxicity. Upon NP exposure, ROS generation is capable of inducing oxidative DNA damage, strand breaks, protein denaturation, and lipid peroxidation thereby demonstrating the mutagenic and carcinogenic characteristics associated with NP. Excess free radical production leads to mitochondrial membrane damage causing necrosis and cell death. Phagocytes including neutrophils and macrophages generate massive ROS upon incomplete phagocytosis of NP through the NADPH-oxidase enzyme system whereas NP-induced ROS triggers an inflammatory cascade of chemokine and cytokine expression via activation of cell signaling pathways such as MAPK, NF- $\kappa$ B, Akt, and RTK. Furthermore, oxidative stress mediated stimulation of these cellular mechanisms results in transcription of genes responsible for fibrosis, EMT, and carcinogenesis. NP-elicited ROS is at the center stage for majority of the ensuing adverse outcomes.

## Abbreviations

ROS:	Reactive oxygen species
NP:	Nanoparticles
NM:	Nanomaterials
RNS:	Reactive nitrogen species
CNT:	Carbon nanotubes
H <sub>2</sub> O <sub>2</sub> :	Hydrogen peroxide
O <sub>2</sub> <sup>•-</sup> :	Superoxide anion
OH <sup>•</sup> :	Hydroxyl radical
<sup>1</sup> O <sub>2</sub> :	Singlet oxygen
HOCl:	Hypochlorous acid
ONOO <sup>-</sup> :	Peroxyne nitrite
AM:	Alveolar macrophages
NADPH:	Nicotinamide adenine dinucleotide phosphate
Nrf2:	Nuclear factor (erythroid-derived 2)-like 2

MAPK:	Mitogen activated protein kinase
NF- $\kappa$ B:	Nuclear factor kappa-light-chain enhancer of activated B cells
iNOS:	Inducible nitric oxide synthase
IL-1 $\beta$ :	Interleukin-1beta
ERKs:	Extracellular signal-related kinases
GSH:	Glutathione
GSSG:	Glutathione disulfide
8-OHdG:	8-Hydroxyl-2'-deoxyguanosine
AP-1:	Activator protein-1
STAT-1:	Signal transducer and activator of transcription-1
RTK:	Receptor tyrosine kinases
PTP:	Protein tyrosine phosphatases
HARN:	High aspect ratio nanomaterials
PARP:	Poly-ADP-ribose polymerase

TNF- $\alpha$ : Tumor necrosis factor-alpha  
 TGF- $\beta$ : Transforming growth factor-beta  
 EMT: Epithelial-mesenchymal transition.

## Disclaimer

Research findings and conclusions are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

## Conflict of Interests

The authors report no conflict of interests.

## Acknowledgments

This work is supported by the National Institutes of Health Grant R01-HL076340 and by the National Science Foundation Grant EPS-1003907.

## References

- [1] A. D. Maynard, P. A. Baron, M. Foley, A. A. Shvedova, E. R. Kisin, and V. Castranova, "Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material," *Journal of Toxicology and Environmental Health A*, vol. 67, no. 1, pp. 87–107, 2004.
- [2] K. Donaldson, F. A. Murphy, R. Duffin, and C. A. Poland, "Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma," *Particle and Fibre Toxicology*, vol. 7, article 5, 2010.
- [3] A. Nel, T. Xia, L. Mädler, and N. Li, "Toxic potential of materials at the nanolevel," *Science*, vol. 311, no. 5761, pp. 622–627, 2006.
- [4] Y. Huang, C. Wu, and R. Aronstam, "Toxicity of transition metal oxide nanoparticles: recent insights from in vitro Studies," *Materials*, vol. 3, no. 10, pp. 4842–4859, 2010.
- [5] G. M. Stella, "Carbon nanotubes and pleural damage: perspectives of nanosafety in the light of asbestos experience," *Biointerphases*, vol. 6, no. 2, pp. P1–P17, 2011.
- [6] A. A. Shvedova, A. Pietroiusti, B. Fadeel, and V. E. Kagan, "Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress," *Toxicology and Applied Pharmacology*, vol. 261, no. 2, pp. 121–133, 2012.
- [7] M. Pojlak-Blazi, M. Jaganjac, and N. Zarkovic, "Cell oxidative stress: risk of metal nanoparticles," in *Handbook of Nanophysics: Nanomedicine and Nanorobotics*, pp. 1–17, CRC Press, New York, NY, USA, 2010.
- [8] Y. Ju-Nam and J. R. Lead, "Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications," *Science of the Total Environment*, vol. 400, no. 1–3, pp. 396–414, 2008.
- [9] N. Li, T. Xia, and A. E. Nel, "The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles," *Free Radical Biology and Medicine*, vol. 44, no. 9, pp. 1689–1699, 2008.
- [10] V. Stone, H. Johnston, and M. J. D. Clift, "Air pollution, ultrafine and nanoparticle toxicology: cellular and molecular interactions," *IEEE Transactions on Nanobioscience*, vol. 6, no. 4, pp. 331–340, 2007.
- [11] H. J. Johnston, G. Hutchison, F. M. Christensen, S. Peters, S. Hankin, and V. Stone, "A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity," *Critical Reviews in Toxicology*, vol. 40, no. 4, pp. 328–346, 2010.
- [12] J. J. Li, S. Muralikrishnan, C. T. Ng, L. Y. Yung, and B. H. Bay, "Nanoparticle-induced pulmonary toxicity," *Experimental Biology and Medicine*, vol. 235, no. 9, pp. 1025–1033, 2010.
- [13] Z. Zhang, A. Berg, H. Levanon, R. W. Fessenden, and D. Meisel, "On the interactions of free radicals with gold nanoparticles," *Journal of the American Chemical Society*, vol. 125, no. 26, pp. 7959–7963, 2003.
- [14] I. M. Kennedy, D. Wilson, and A. I. Barakat, "Uptake and inflammatory effects of nanoparticles in a human vascular endothelial cell line," *Research Report*, no. 136, pp. 3–32, 2009.
- [15] H. Lee, D. Shin, H. Song et al., "Nanoparticles up-regulate tumor necrosis factor- $\alpha$  and CXCL8 via reactive oxygen species and mitogen-activated protein kinase activation," *Toxicology and Applied Pharmacology*, vol. 238, no. 2, pp. 160–169, 2009.
- [16] C. Buzea, I. I. Pacheco, and K. Robbie, "Nanomaterials and nanoparticles: sources and toxicity," *Biointerphases*, vol. 2, no. 4, pp. MR17–MR71, 2007.
- [17] B. Fubini and A. Hubbard, "Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis," *Free Radical Biology and Medicine*, vol. 34, no. 12, pp. 1507–1516, 2003.
- [18] X. He, S. Young, D. Schwegler-Berry, W. P. Chisholm, J. E. Fernback, and Q. Ma, "Multiwalled carbon nanotubes induce a fibrogenic response by stimulating reactive oxygen species production, activating NF- $\kappa$ B signaling, and promoting fibroblast-to-myofibroblast transformation," *Chemical Research in Toxicology*, vol. 24, no. 12, pp. 2237–2248, 2011.
- [19] L. Wang, R. R. Mercer, Y. Rojanasakul et al., "Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts," *Journal of Toxicology and Environmental Health A*, vol. 73, no. 5–6, pp. 410–422, 2010.
- [20] V. Vallyathan and X. Shi, "The role of oxygen free radicals in occupational and environmental lung diseases," *Environmental Health Perspectives*, vol. 105, supplement 1, pp. 165–177, 1997.
- [21] V. J. Thannickal and B. L. Fanburg, "Reactive oxygen species in cell signaling," *American Journal of Physiology*, vol. 279, no. 6, pp. L1005–L1028, 2000.
- [22] L. Risom, P. Møller, and S. Loft, "Oxidative stress-induced DNA damage by particulate air pollution," *Mutation Research*, vol. 592, no. 1–2, pp. 119–137, 2005.
- [23] C. Huang, R. S. Aronstam, D. Chen, and Y. Huang, "Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles," *Toxicology in Vitro*, vol. 24, no. 1, pp. 45–55, 2010.
- [24] H. Sies, "Oxidative stress: introduction," in *Oxidative Stress Oxidants and Antioxidants*, H. Sies, Ed., pp. 15–22, Academic Press, London, UK, 1991.
- [25] A. M. Knaapen, P. J. A. Borm, C. Albrecht, and R. P. F. Schins, "Inhaled particles and lung cancer, part A: mechanisms," *International Journal of Cancer*, vol. 109, no. 6, pp. 799–809, 2004.
- [26] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, and M. Mazur, "Free radicals, metals and antioxidants in oxidative stress-induced cancer," *Chemico-Biological Interactions*, vol. 160, no. 1, pp. 1–40, 2006.
- [27] K. Donaldson and C. L. Tran, "Inflammation caused by particles and fibers," *Inhalation Toxicology*, vol. 14, no. 1, pp. 5–27, 2002.

- [28] K. Donaldson, V. Stone, A. Clouter, L. Renwick, and W. MacNee, "Ultrafine particles," *Occupational and Environmental Medicine*, vol. 58, no. 3, pp. 211–216, 2001.
- [29] K. Donaldson, V. Stone, C. L. Tran, W. Kreyling, and P. J. A. Borm, "Nanotoxicology," *Occupational and Environmental Medicine*, vol. 61, no. 9, pp. 727–728, 2004.
- [30] A. Nel, "Air pollution-related illness: effects of particles," *Science*, vol. 308, no. 5723, pp. 804–806, 2005.
- [31] J. C. Bonner, "Lung fibrotic responses to particle exposure," *Toxicologic Pathology*, vol. 35, no. 1, pp. 148–153, 2007.
- [32] R. P. F. Schins, "Mechanisms of genotoxicity of particles and fibers," *Inhalation Toxicology*, vol. 14, no. 1, pp. 57–78, 2002.
- [33] G. Oberdörster, A. Maynard, K. Donaldson et al., "Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy," *Particle and Fibre Toxicology*, vol. 2, article 8, 2005.
- [34] M. R. Wilson, J. H. Lightbody, K. Donaldson, J. Sales, and V. Stone, "Interactions between ultrafine particles and transition metals in vivo and in vitro," *Toxicology and Applied Pharmacology*, vol. 184, no. 3, pp. 172–179, 2002.
- [35] S. D. Aust, C. F. Chignell, T. M. Bray, B. Kalyanaraman, and R. P. Mason, "Free radicals in toxicology," *Toxicology and Applied Pharmacology*, vol. 120, no. 2, pp. 168–178, 1993.
- [36] K. R. Smith, L. R. Klei, and A. Barchowsky, "Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells," *American Journal of Physiology*, vol. 280, no. 3, pp. L442–L449, 2001.
- [37] C. Sioutas, R. J. Delfino, and M. Singh, "Exposure assessment for atmospheric Ultrafine Particles (UFPs) and implications in epidemiologic research," *Environmental Health Perspectives*, vol. 113, no. 8, pp. 947–955, 2005.
- [38] T. Xia, M. Kovochich, J. Brant et al., "Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm," *Nano Letters*, vol. 6, no. 8, pp. 1794–1807, 2006.
- [39] T. Coccini, S. Barni, R. Vaccarone, P. Mustarelli, L. Manzo, and E. Roda, "Pulmonary toxicity of instilled cadmium-doped silica nanoparticles during acute and subacute stages in rats," *Histology and Histopathology*, vol. 28, no. 2, pp. 195–209, 2013.
- [40] V. Stone, J. Shaw, D. M. Brown, W. Macnee, S. P. Faux, and K. Donaldson, "The role of oxidative stress in the prolonged inhibitory effect of ultrafine carbon black on epithelial cell function," *Toxicology in Vitro*, vol. 12, no. 6, pp. 649–659, 1998.
- [41] S. K. Sohaebuddin, P. T. Thevenot, D. Baker, J. W. Eaton, and L. Tang, "Nanomaterial cytotoxicity is composition, size, and cell type dependent," *Particle and Fibre Toxicology*, vol. 7, article 22, 2010.
- [42] V. K. Raghunathan, M. Devey, S. Hawkins et al., "Influence of particle size and reactive oxygen species on cobalt chrome nanoparticle-mediated genotoxicity," *Biomaterials*, vol. 34, no. 14, pp. 3559–3570, 2013.
- [43] K. E. Driscoll, B. W. Howard, J. M. Carter, Y. M. W. Janssen, B. T. Mossman, and R. J. Isfort, "Mitochondrial-derived oxidants and quartz activation of chemokine gene expression," *Advances in Experimental Medicine and Biology*, vol. 500, pp. 489–496, 2001.
- [44] B. Fadeel and V. E. Kagan, "Apoptosis and macrophage clearance of neutrophils: regulation by reactive oxygen species," *Redox Report*, vol. 8, no. 3, pp. 143–150, 2003.
- [45] A. Deshpande, P. K. Narayanan, and B. E. Lehnert, "Silica-induced generation of extracellular factor(s) increases reactive oxygen species in human bronchial epithelial cells," *Toxicological Sciences*, vol. 67, no. 2, pp. 275–283, 2002.
- [46] I. Berg, T. Schluter, and G. Gercken, "Increase of bovine alveolar macrophage superoxide anion and hydrogen peroxide release by dusts of different origin," *Journal of Toxicology and Environmental Health*, vol. 39, no. 3, pp. 341–354, 1993.
- [47] W. A. Pryor, K. Stone, C. E. Cross, L. Machlin, and L. Packer, "Oxidants in cigarette smoke: radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite," *Annals of the New York Academy of Sciences*, vol. 686, pp. 12–28, 1993.
- [48] V. Castranova, L. J. Huffman, D. J. Judy et al., "Enhancement of nitric oxide production by pulmonary cells following silica exposure," *Environmental Health Perspectives*, vol. 106, supplement 5, pp. 1165–1169, 1998.
- [49] J. M. Carter and K. E. Driscoll, "The role of inflammation, oxidative stress, and proliferation in silica-induced lung disease: a species comparison," *Journal of Environmental Pathology, Toxicology and Oncology*, vol. 20, supplement 1, pp. 33–43, 2001.
- [50] Y. Hsin, C. Chen, S. Huang, T. Shih, P. Lai, and P. J. Chueh, "The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells," *Toxicology Letters*, vol. 179, no. 3, pp. 130–139, 2008.
- [51] P. J. A. Borm, D. Robbins, S. Haubold et al., "The potential risks of nanomaterials: a review carried out for ECETOC," *Particle and Fibre Toxicology*, vol. 3, article 11, 2006.
- [52] H. Eom and J. Choi, "p38 MAPK activation, DNA damage, cell cycle arrest and apoptosis as mechanisms of toxicity of silver nanoparticles in Jurkat T cells," *Environmental Science and Technology*, vol. 44, no. 21, pp. 8337–8342, 2010.
- [53] G. Lenaz, "The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology," *IUBMB Life*, vol. 52, no. 3–5, pp. 159–164, 2001.
- [54] J. F. Turrens, "Mitochondrial formation of reactive oxygen species," *Journal of Physiology*, vol. 552, no. 2, pp. 335–344, 2003.
- [55] J. Boonstra and J. A. Post, "Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells," *Gene*, vol. 337, pp. 1–13, 2004.
- [56] L. Wang, L. Bowman, Y. Lu et al., "Essential role of p53 in silica-induced apoptosis," *American Journal of Physiology*, vol. 288, no. 3, pp. L488–L496, 2005.
- [57] Y. Shi, F. Wang, J. He, S. Yadav, and H. Wang, "Titanium dioxide nanoparticles cause apoptosis in BEAS-2B cells through the caspase 8/t-Bid-independent mitochondrial pathway," *Toxicology Letters*, vol. 196, no. 1, pp. 21–27, 2010.
- [58] P. Manna, M. Ghosh, J. Ghosh, J. Das, and P. C. Sil, "Contribution of nano-copper particles to in vivo liver dysfunction and cellular damage: role of I $\kappa$ B $\alpha$ /NF- $\kappa$ B, MAPKs and mitochondrial signal," *Nanotoxicology*, vol. 6, no. 1, pp. 1–21, 2012.
- [59] X. Q. Zhang, L. H. Yin, M. Tang, and Y. P. Pu, "ZnO, TiO<sub>2</sub>, SiO<sub>2</sub>, and Al<sub>2</sub>O<sub>3</sub> nanoparticles-induced toxic effects on human fetal lung fibroblasts," *Biomedical and Environmental Sciences*, vol. 24, no. 6, pp. 661–669, 2011.
- [60] M. Poljak-Blazi, M. Jaganjac, M. Mustapic, N. Pivac, and D. Muck-Seler, "Acute immunomodulatory effects of iron polyisomaltoate in rats," *Immunobiology*, vol. 214, no. 2, pp. 121–128, 2009.
- [61] S. Naqvi, M. Samim, M. Z. Abdin et al., "Concentration-dependent toxicity of iron oxide nanoparticles mediated by increased oxidative stress," *International Journal of Nanomedicine*, vol. 5, no. 1, pp. 983–989, 2010.



- [62] I. Pujalté, I. Passagne, B. Brouillaud et al., "Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells," *Particle and Fibre Toxicology*, vol. 8, article 10, 2011.
- [63] H. A. Jeng and J. Swanson, "Toxicity of metal oxide nanoparticles in mammalian cells," *Journal of Environmental Science and Health A*, vol. 41, no. 12, pp. 2699–2711, 2006.
- [64] E. Park, J. Choi, Y. Park, and K. Park, "Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells," *Toxicology*, vol. 245, no. 1-2, pp. 90–100, 2008.
- [65] A. Kumar, A. K. Pandey, S. S. Singh, R. Shanker, and A. Dhawan, "Engineered ZnO and TiO<sub>2</sub> nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*," *Free Radical Biology and Medicine*, vol. 51, no. 10, pp. 1872–1881, 2011.
- [66] I. Kim, M. Baek, and S. Choi, "Comparative cytotoxicity of Al<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub>, TiO<sub>2</sub> and ZnO nanoparticles to human lung cells," *Journal of Nanoscience and Nanotechnology*, vol. 10, no. 5, pp. 3453–3458, 2010.
- [67] S. Kawanishi, Y. Hiraku, M. Murata, and S. Oikawa, "The role of metals in site-specific DNA damage with reference to carcinogenesis," *Free Radical Biology and Medicine*, vol. 32, no. 9, pp. 822–832, 2002.
- [68] H. Shi, L. G. Hudson, and K. J. Liu, "Oxidative stress and apoptosis in metal ion-induced carcinogenesis," *Free Radical Biology and Medicine*, vol. 37, no. 5, pp. 582–593, 2004.
- [69] H. Wiseman and B. Halliwell, "Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer," *Biochemical Journal*, vol. 313, part 1, pp. 17–29, 1996.
- [70] H. Xie, M. M. Mason, and J. P. Wise Sr., "Genotoxicity of metal nanoparticles," *Reviews on Environmental Health*, vol. 26, no. 4, pp. 251–268, 2011.
- [71] A. Pilger and H. W. Rüdiger, "8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures," *International Archives of Occupational and Environmental Health*, vol. 80, no. 1, pp. 1–15, 2006.
- [72] A. Valavanidis, T. Vlachogianni, and C. Fiotakis, "8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis," *Journal of Environmental Science and Health C*, vol. 27, no. 2, pp. 120–139, 2009.
- [73] K. Inoue, H. Takano, R. Yanagisawa et al., "Effects of airway exposure to nanoparticles on lung inflammation induced by bacterial endotoxin in mice," *Environmental Health Perspectives*, vol. 114, no. 9, pp. 1325–1330, 2006.
- [74] K. E. Eblin, M. E. Bowen, D. W. Cromey et al., "Arsenite and monomethylarsonous acid generate oxidative stress response in human bladder cell culture," *Toxicology and Applied Pharmacology*, vol. 217, no. 1, pp. 7–14, 2006.
- [75] M. Song, Y. Li, H. Kasai, and K. Kawai, "Metal nanoparticle-induced micronuclei and oxidative DNA damage in mice," *Journal of Clinical Biochemistry and Nutrition*, vol. 50, no. 3, pp. 211–216, 2012.
- [76] P. J. Howden and S. P. Faux, "Fibre-induced lipid peroxidation leads to DNA adduct formation in *Salmonella typhimurium* TA104 and rat lung fibroblasts," *Carcinogenesis*, vol. 17, no. 3, pp. 413–419, 1996.
- [77] R. K. Shukla, V. Sharma, A. K. Pandey, S. Singh, S. Sultana, and A. Dhawan, "ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells," *Toxicology in Vitro*, vol. 25, no. 1, pp. 231–241, 2011.
- [78] D. Napierska, V. Rabolli, L. C. J. Thomassen et al., "Oxidative stress induced by pure and iron-doped amorphous silica nanoparticles in subtoxic conditions," *Chemical Research in Toxicology*, vol. 25, no. 4, pp. 828–837, 2012.
- [79] M. L. Turski and D. J. Thiele, "New roles for copper metabolism in cell proliferation, signaling, and disease," *The Journal of Biological Chemistry*, vol. 284, no. 2, pp. 717–721, 2009.
- [80] I. Rahman, S. K. Biswas, L. A. Jimenez, M. Torres, and H. J. Forman, "Glutathione, stress responses, and redox signaling in lung inflammation," *Antioxidants and Redox Signaling*, vol. 7, no. 1-2, pp. 42–59, 2005.
- [81] G. M. Habib, Z. Shi, and M. W. Lieberman, "Glutathione protects cells against arsenite-induced toxicity," *Free Radical Biology and Medicine*, vol. 42, no. 2, pp. 191–201, 2007.
- [82] K. Rahman, "Studies on free radicals, antioxidants, and co-factors," *Clinical Interventions in Aging*, vol. 2, no. 2, pp. 219–236, 2007.
- [83] I. Fenoglio, I. Corazzari, C. Francia, S. Bodoardo, and B. Fubini, "The oxidation of glutathione by cobalt/tungsten carbide contributes to hard metal-induced oxidative stress," *Free Radical Research*, vol. 42, no. 8, pp. 737–745, 2008.
- [84] C. Stambe, R. C. Atkins, G. H. Tesch, T. Masaki, G. F. Schreiner, and D. J. Nikolic-Paterson, "The role of p38alpha mitogen-activated protein kinase activation in renal fibrosis," *Journal of the American Society of Nephrology*, vol. 15, no. 2, pp. 370–379, 2004.
- [85] E. Park, J. Yoon, K. Choi, J. Yi, and K. Park, "Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation," *Toxicology*, vol. 260, no. 1-3, pp. 37–46, 2009.
- [86] C. Moon, H. Park, Y. Choi, E. Park, V. Castranova, and J. L. Kang, "Pulmonary inflammation after intraperitoneal administration of ultrafine titanium dioxide (TiO<sub>2</sub>) at rest or in lungs primed with lipopolysaccharide," *Journal of Toxicology and Environmental Health A*, vol. 73, no. 5-6, pp. 396–409, 2010.
- [87] M. A. Maurer-Jones, Y. Lin, and C. L. Haynes, "Functional assessment of metal oxide nanoparticle toxicity in immune cells," *ACS Nano*, vol. 4, no. 6, pp. 3363–3373, 2010.
- [88] M. Zhu, W. Feng, Y. Wang et al., "Particokinetics and extra-pulmonary translocation of intratracheally instilled ferric oxide nanoparticles in rats and the potential health risk assessment," *Toxicological Sciences*, vol. 107, no. 2, pp. 342–351, 2009.
- [89] C. Lam, J. T. James, R. McCluskey, and R. L. Hunter, "Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation," *Toxicological Sciences*, vol. 77, no. 1, pp. 126–134, 2004.
- [90] A. A. Shvedova, E. R. Kisin, R. Mercer et al., "Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice," *American Journal of Physiology*, vol. 289, no. 5, pp. L698–L708, 2005.
- [91] A. A. Shvedova, E. Kisin, A. R. Murray et al., "Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis," *American Journal of Physiology*, vol. 295, no. 4, pp. L552–L565, 2008.
- [92] K. Pulskamp, S. Diabaté, and H. F. Krug, "Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants," *Toxicology Letters*, vol. 168, no. 1, pp. 58–74, 2007.
- [93] M. Pacurari, X. J. Yin, J. Zhao et al., "Raw single-wall carbon nanotubes induce oxidative stress and activate MAPKs, AP-1,



- NF- $\kappa$ B, and Akt in normal and malignant human mesothelial cells," *Environmental Health Perspectives*, vol. 116, no. 9, pp. 1211–1217, 2008.
- [94] J. L. Ingram, A. B. Rice, J. Santos, B. Van Houten, and J. C. Bonner, "Vanadium-induced HB-EGF expression in human lung fibroblasts is oxidant dependent and requires MAP kinases," *American Journal of Physiology*, vol. 284, no. 5, pp. L774–L782, 2003.
- [95] J. M. Antonini, "Health effects of welding," *Critical Reviews in Toxicology*, vol. 33, no. 1, pp. 61–103, 2003.
- [96] P. D. Blanc, H. A. Boushey, H. Wong, S. F. Wintermeyer, and M. S. Bernstein, "Cytokines in metal fume fever," *American Review of Respiratory Disease*, vol. 147, no. 1, pp. 134–138, 1993.
- [97] M. D. Taylor, J. R. Roberts, S. S. Leonard, X. Shi, and J. M. Antonini, "Effects of welding fumes of differing composition and solubility on free radical production and acute lung injury and inflammation in rats," *Toxicological Sciences*, vol. 75, no. 1, pp. 181–191, 2003.
- [98] M. Genestra, "Oxyl radicals, redox-sensitive signalling cascades and antioxidants," *Cellular Signalling*, vol. 19, no. 9, pp. 1807–1819, 2007.
- [99] J. Ye, X. Zhang, H. A. Young, Y. Mao, and X. Shi, "Chromium(VI)-induced nuclear factor- $\kappa$ B activation in intact cells via free radical reactions," *Carcinogenesis*, vol. 16, no. 10, pp. 2401–2405, 1995.
- [100] R. G. Allen and M. Tresini, "Oxidative stress and gene regulation," *Free Radical Biology and Medicine*, vol. 28, no. 3, pp. 463–499, 2000.
- [101] J. D. Byrne and J. A. Baugh, "The significance of nanoparticles in particle-induced pulmonary fibrosis," *McGill Journal of Medicine*, vol. 11, no. 1, pp. 43–50, 2008.
- [102] A. R. Murray, E. R. Kisin, A. V. Tkach et al., "Factoring-in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos," *Particle and Fibre Toxicology*, vol. 9, article 10, 2012.
- [103] A. K. Hubbard, C. R. Timblin, A. Shukla, M. Rincón, and B. T. Mossman, "Activation of NF- $\kappa$ B-dependent gene expression by silica in lungs of luciferase reporter mice," *American Journal of Physiology*, vol. 282, no. 5, pp. L968–L975, 2002.
- [104] M. Ding, X. Shi, Y. Lu et al., "Induction of activator protein-1 through reactive oxygen species by crystalline silica in JB6 cells," *The Journal of Biological Chemistry*, vol. 276, no. 12, pp. 9108–9114, 2001.
- [105] K. Z. Guyton, Y. Liu, M. Gorospe, Q. Xu, and N. J. Holbrook, "Activation of mitogen-activated protein kinase by H<sub>2</sub>O<sub>2</sub>: role in cell survival following oxidant injury," *The Journal of Biological Chemistry*, vol. 271, no. 8, pp. 4138–4142, 1996.
- [106] C. Tournier, G. Thomas, J. Pierre, C. Jacquemin, M. Pierre, and B. Saunier, "Mediation by arachidonic acid metabolites of the H<sub>2</sub>O<sub>2</sub>-induced stimulation of mitogen-activated protein kinases (extracellular-signal-regulated kinase and c-Jun NH<sub>2</sub>-terminal kinase)," *European Journal of Biochemistry*, vol. 244, no. 2, pp. 587–595, 1997.
- [107] Y. Son, Y. Cheong, N. Kim, H. Chung, D. G. Kang, and H. Pae, "Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways?" *Journal of Signal Transduction*, vol. 2011, Article ID 792639, 6 pages, 2011.
- [108] D. M. Barrett, S. M. Black, H. Todor, R. K. Schmidt-Ullrich, K. S. Dawson, and R. B. Mikkelsen, "Inhibition of protein-tyrosine phosphatases by mild oxidative stresses is dependent on S-nitrosylation," *The Journal of Biological Chemistry*, vol. 280, no. 15, pp. 14453–14461, 2005.
- [109] Y. Kim, W. Reed, W. Wu, P. A. Bromberg, L. M. Graves, and J. M. Samet, "Zn<sup>2+</sup>-induced IL-8 expression involves AP-1, JNK, and ERK activities in human airway epithelial cells," *American Journal of Physiology*, vol. 290, no. 5, pp. L1028–L1035, 2006.
- [110] T. L. Tal, L. M. Graves, R. Silbajoris, P. A. Bromberg, W. Wu, and J. M. Samet, "Inhibition of protein tyrosine phosphatase activity mediates epidermal growth factor receptor signaling in human airway epithelial cells exposed to Zn<sup>2+</sup>," *Toxicology and Applied Pharmacology*, vol. 214, no. 1, pp. 16–23, 2006.
- [111] F. Esposito, G. Chirico, N. M. Gesualdi et al., "Protein kinase B activation by reactive oxygen species is independent of tyrosine kinase receptor phosphorylation and requires Src activity," *The Journal of Biological Chemistry*, vol. 278, no. 23, pp. 20828–20834, 2003.
- [112] K. Balamurugan, R. Rajaram, T. Ramasami, and S. Narayanan, "Chromium(III)-induced apoptosis of lymphocytes: death decision by ROS and Src-family tyrosine kinases," *Free Radical Biology and Medicine*, vol. 33, no. 12, pp. 1622–1640, 2002.
- [113] British Standard Institute (BSI), "Nanotechnologies—part 2: guide to safe handling and disposal of manufactured nanomaterials," Tech. Rep. PD, 6699-2, British Standard Institute (BSI), London, UK, 2007.
- [114] T. Xia, M. Kovochich, M. Liong, J. I. Zink, and A. E. Nel, "Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways," *ACS Nano*, vol. 2, no. 1, pp. 85–96, 2008.
- [115] A. Le Goff, M. Holzinger, and S. Cosnier, "Enzymatic biosensors based on SWCNT-conducting polymer electrodes," *Analyst*, vol. 136, no. 7, pp. 1279–1287, 2011.
- [116] D. B. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. A. M. Reynolds, and T. R. Webb, "Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats," *Toxicological Sciences*, vol. 77, no. 1, pp. 117–125, 2004.
- [117] J. C. Bonner, "The epidermal growth factor receptor at the crossroads of airway remodeling," *American Journal of Physiology*, vol. 283, no. 3, pp. L528–L530, 2002.
- [118] N. Azad, A. K. Iyer, L. Wang, Y. Liu, Y. Lu, and Y. Rojanasakul, "Reactive oxygen species-mediated p38 MAPK regulates carbon nanotube-induced fibrogenic and angiogenic responses," *Nanotoxicology*, vol. 7, no. 2, pp. 157–168, 2012.
- [119] S. K. Manna, S. Sarkar, J. Barr et al., "Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor- $\kappa$ B in human keratinocytes," *Nano Letters*, vol. 5, no. 9, pp. 1676–1684, 2005.
- [120] A. A. Shvedova, A. A. Kapralov, W. H. Feng et al., "Impaired clearance and enhanced pulmonary inflammatory/fibrotic response to carbon nanotubes in myeloperoxidase-deficient mice," *PLoS ONE*, vol. 7, no. 3, Article ID e30923, 2012.
- [121] A. R. Reddy, D. R. Krishna, Y. N. Reddy, and V. Himabindu, "Translocation and extra pulmonary toxicities of multi wall carbon nanotubes in rats," *Toxicology Mechanisms and Methods*, vol. 20, no. 5, pp. 267–272, 2010.
- [122] L. A. Mitchell, J. Gao, R. V. Wal, A. Gigliotti, S. W. Burchiel, and J. D. McDonald, "Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes," *Toxicological Sciences*, vol. 100, no. 1, pp. 203–214, 2007.
- [123] S. Clichici, A. R. Biris, F. Tabaran, and A. Filip, "Transient oxidative stress and inflammation after intraperitoneal administration of multiwalled carbon nanotubes functionalized with single strand DNA in rats," *Toxicology and Applied Pharmacology*, vol. 259, no. 3, pp. 281–292, 2012.

- [124] K. Donaldson and C. A. Poland, "Inhaled nanoparticles and lung cancer—what we can learn from conventional particle toxicology," *Swiss Medical Weekly*, vol. 142, Article ID w13547, 2012.
- [125] M. Pacurari, Y. Qian, D. W. Porter et al., "Multi-walled carbon nanotube-induced gene expression in the mouse lung: association with lung pathology," *Toxicology and Applied Pharmacology*, vol. 255, no. 1, pp. 18–31, 2011.
- [126] M. C. Jaurand, "Mechanisms of fiber-induced genotoxicity," *Environmental Health Perspectives*, vol. 105, supplement 5, pp. 1073–1084, 1997.
- [127] M. F. Jaurand, A. Renier, and J. Daubriac, "Mesothelioma: do asbestos and carbon nanotubes pose the same health risk?" *Particle and Fibre Toxicology*, vol. 6, article 16, 2009.
- [128] A. K. Patlolla, S. M. Hussain, J. J. Schlager, S. Patlolla, and P. B. Tchounwou, "Comparative study of the clastogenicity of functionalized and nonfunctionalized multiwalled carbon nanotubes in bone marrow cells of Swiss-Webster mice," *Environmental Toxicology*, vol. 25, no. 6, pp. 608–621, 2010.
- [129] A. Patlolla, B. Knighten, and P. Tchounwou, "Multi-walled carbon nanotubes induce cytotoxicity, genotoxicity and apoptosis in normal human dermal fibroblast cells," *Ethnicity & Disease*, vol. 20, supplement 1, pp. 65–72, 2010.
- [130] E. R. Kisin, A. R. Murray, L. Sargent et al., "Genotoxicity of carbon nanofibers: are they potentially more or less dangerous than carbon nanotubes or asbestos?" *Toxicology and Applied Pharmacology*, vol. 252, no. 1, pp. 1–10, 2011.
- [131] E. R. Kisin, A. R. Murray, M. J. Keane et al., "Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells," *Journal of Toxicology and Environmental Health A*, vol. 70, no. 24, pp. 2071–2079, 2007.
- [132] D. van Berlo, M. J. Clift, C. Albrecht, and R. P. Schins, "Carbon nanotubes: an insight into the mechanisms of their potential genotoxicity," *Swiss Medical Weekly*, vol. 142, Article ID w13698, 2012.
- [133] Y. Guo, J. Zhang, Y. Zheng, J. Yang, and X. Zhu, "Cytotoxic and genotoxic effects of multi-wall carbon nanotubes on human umbilical vein endothelial cells in vitro," *Mutation Research*, vol. 721, no. 2, pp. 184–191, 2011.
- [134] A. R. N. Reddy, M. V. Rao, D. R. Krishna, V. Himabindu, and Y. N. Reddy, "Evaluation of oxidative stress and anti-oxidant status in rat serum following exposure of carbon nanotubes," *Regulatory Toxicology and Pharmacology*, vol. 59, no. 2, pp. 251–257, 2011.
- [135] F. Zhou, D. Xing, B. Wu, S. Wu, Z. Ou, and W. R. Chen, "New insights of transmembranal mechanism and subcellular localization of noncovalently modified single-walled carbon nanotubes," *Nano Letters*, vol. 10, no. 5, pp. 1677–1681, 2010.
- [136] P. Ravichandran, S. Baluchamy, B. Sadanandan et al., "Multi-walled carbon nanotubes activate NF- $\kappa$ B and AP-1 signaling pathways to induce apoptosis in rat lung epithelial cells," *Apoptosis*, vol. 15, no. 12, pp. 1507–1516, 2010.
- [137] J. Muller, I. Decordier, P. H. Hoet et al., "Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells," *Carcinogenesis*, vol. 29, no. 2, pp. 427–433, 2008.
- [138] T. Kisseleva and D. A. Brenner, "Mechanisms of fibrogenesis," *Experimental Biology and Medicine*, vol. 233, no. 2, pp. 109–122, 2008.
- [139] X. He, S. Young, J. E. Fernback, and Q. Ma, "Single-walled carbon nanotubes induce fibrogenic effect by disturbing mitochondrial oxidative stress and activating NF- $\kappa$ B signaling," *Journal of Clinical Toxicology*, supplement S5, article 005, 2012.
- [140] C. R. Keenan, R. Goth-Goldstein, D. Lucas, and D. L. Sedlak, "Oxidative stress induced by zero-valent iron nanoparticles and Fe(II) in human bronchial epithelial cells," *Environmental Science and Technology*, vol. 43, no. 12, pp. 4555–4560, 2009.
- [141] P. L. Apopa, Y. Qian, R. Shao et al., "Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling," *Particle and Fibre Toxicology*, vol. 6, article 1, 2009.
- [142] A. R. Murray, E. Kisin, A. Inman et al., "Oxidative stress and dermal toxicity of iron oxide nanoparticles in vitro," *Cell Biochemistry and Biophysics*. In press.
- [143] M. Ahamed, M. A. Siddiqui, M. J. Akhtar, I. Ahmad, A. B. Pant, and H. A. Alhadlaq, "Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells," *Biochemical and Biophysical Research Communications*, vol. 396, no. 2, pp. 578–583, 2010.
- [144] H. L. Karlsson, J. Gustafsson, P. Cronholm, and L. Möller, "Size-dependent toxicity of metal oxide particles—a comparison between nano- and micrometer size," *Toxicology Letters*, vol. 188, no. 2, pp. 112–118, 2009.
- [145] B. Fahmy and S. A. Cormier, "Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells," *Toxicology in Vitro*, vol. 23, no. 7, pp. 1365–1371, 2009.
- [146] R. Lei, C. Wu, B. Yang et al., "Integrated metabolomic analysis of the nano-sized copper particle-induced hepatotoxicity and nephrotoxicity in rats: a rapid in vivo screening method for nanotoxicity," *Toxicology and Applied Pharmacology*, vol. 232, no. 2, pp. 292–301, 2008.
- [147] J. Y. Ma, H. Zhao, R. R. Mercer et al., "Cerium oxide nanoparticle-induced pulmonary inflammation and alveolar macrophage functional change in rats," *Nanotoxicology*, vol. 5, no. 3, pp. 312–325, 2011.
- [148] H. Eom and J. Choi, "Oxidative stress of CeO<sub>2</sub> nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B," *Toxicology Letters*, vol. 187, no. 2, pp. 77–83, 2009.
- [149] W. Lin, Y. Huang, X. Zhou, and Y. Ma, "Toxicity of cerium oxide nanoparticles in human lung cancer cells," *International Journal of Toxicology*, vol. 25, no. 6, pp. 451–457, 2006.
- [150] T. Xia, M. Kovochich, M. Liong et al., "Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties," *ACS Nano*, vol. 2, no. 10, pp. 2121–2134, 2008.
- [151] B. De Berardis, G. Civitelli, M. Condello et al., "Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells," *Toxicology and Applied Pharmacology*, vol. 246, no. 3, pp. 116–127, 2010.
- [152] V. Sharma, D. Anderson, and A. Dhawan, "Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2)," *Apoptosis*, vol. 17, no. 8, pp. 852–870, 2012.
- [153] S. Alarifi, D. Ali, S. Alkahtani et al., "Induction of oxidative stress, DNA damage, and apoptosis in a malignant human skin melanoma cell line after exposure to zinc oxide nanoparticles," *International Journal of Nanomedicine*, vol. 8, pp. 983–993, 2013.
- [154] D. Guo, H. Bi, B. Liu, Q. Wu, D. Wang, and Y. Cui, "Reactive oxygen species-induced cytotoxic effects of zinc oxide nanoparticles in rat retinal ganglion cells," *Toxicology in Vitro*, vol. 27, no. 2, pp. 731–738, 2013.
- [155] M. Ahamed, M. J. Akhtar, M. Raja et al., "ZnO nanorod-induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: role of oxidative stress," *Nanomedicine*, vol. 7, no. 6, pp. 904–913, 2011.

- [156] Y. Ye, J. Liu, J. Xu, L. Sun, M. Chen, and M. Lan, "Nano-SiO<sub>2</sub> induces apoptosis via activation of p53 and Bax mediated by oxidative stress in human hepatic cell line," *Toxicology in Vitro*, vol. 24, no. 3, pp. 751–758, 2010.
- [157] Y. Ye, J. Liu, M. Chen, L. Sun, and M. Lan, "In vitro toxicity of silica nanoparticles in myocardial cells," *Environmental Toxicology and Pharmacology*, vol. 29, no. 2, pp. 131–137, 2010.
- [158] F. Wang, F. Gao, M. Lan, H. Yuan, Y. Huang, and J. Liu, "Oxidative stress contributes to silica nanoparticle-induced cytotoxicity in human embryonic kidney cells," *Toxicology in Vitro*, vol. 23, no. 5, pp. 808–815, 2009.
- [159] Q. Chen, Y. Xue, and J. Sun, "Kupffer cell-mediated hepatic injury induced by silica nanoparticles in vitro and in vivo," *International Journal of Nanomedicine*, vol. 8, pp. 1129–1140, 2013.
- [160] M. A. Siddiqui, M. Ahamed, J. Ahmad et al., "Nickel oxide nanoparticles induce cytotoxicity, oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin," *Food and Chemical Toxicology*, vol. 50, no. 3–4, pp. 641–647, 2012.
- [161] M. Ahamed, M. J. Akhtar, M. A. Siddiqui et al., "Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells," *Toxicology*, vol. 283, no. 2–3, pp. 101–108, 2011.
- [162] K. C. Yoo, C. H. Yoon, D. Kwon, K. H. Hyun, S. J. Woo, R. K. Kim et al., "Titanium dioxide induces apoptotic cell death through reactive oxygen species-mediated fas upregulation and bax activation," *International Journal of Nanomedicine*, vol. 7, pp. 1203–1214, 2012.
- [163] Q. Saquib, A. A. Al-Khedhairi, M. A. Siddiqui, F. M. Abou-Tarboush, A. Azam, and J. Musarrat, "Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells," *Toxicology in Vitro*, vol. 26, no. 2, pp. 351–361, 2012.
- [164] K. M. Ramkumar, C. Manjula, G. GnanaKumar et al., "Oxidative stress-mediated cytotoxicity and apoptosis induction by TiO<sub>2</sub> nanofibers in HeLa cells," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 81, no. 2, pp. 324–333, 2012.
- [165] A. A. Alshatwi, P. V. Subbarayan, E. Ramesh, A. A. Al-Hazzani, M. A. Alsaif, and A. A. Alwarthan, "Aluminium oxide nanoparticles induce mitochondrial-mediated oxidative stress and alter the expression of antioxidant enzymes in human mesenchymal stem cells," *Food Additives and Contaminants A*, vol. 30, no. 1, pp. 1–10, 2013.
- [166] J. J. Li, D. Hartono, C. Ong, B. Bay, and L. L. Yung, "Autophagy and oxidative stress associated with gold nanoparticles," *Biomaterials*, vol. 31, no. 23, pp. 5996–6003, 2010.
- [167] P. V. AshaRani, G. L. K. Mun, M. P. Hande, and S. Valiyaveetil, "Cytotoxicity and genotoxicity of silver nanoparticles in human cells," *ACS Nano*, vol. 3, no. 2, pp. 279–290, 2009.
- [168] P. Chairuangkitti, S. Lawanprasert, S. Roytrakul, S. Aueviriyavit, D. Phummiratch, K. Kulthong et al., "Silver nanoparticles induce toxicity in A549 cells via ROS-dependent and ROS-independent pathways," *Toxicology in Vitro*, vol. 27, no. 1, pp. 330–338, 2013.
- [169] I. Papageorgiou, C. Brown, R. Schins et al., "The effect of nano- and micron-sized particles of cobalt-chromium alloy on human fibroblasts in vitro," *Biomaterials*, vol. 28, no. 19, pp. 2946–2958, 2007.
- [170] A. A. Shvedova, V. Castranova, E. R. Kisin et al., "Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells," *Journal of Toxicology and Environmental Health A*, vol. 66, no. 20, pp. 1909–1926, 2003.
- [171] C. S. Sharma, S. Sarkar, A. Periyakaruppan et al., "Single-walled carbon nanotubes induces oxidative stress in rat lung epithelial cells," *Journal of Nanoscience and Nanotechnology*, vol. 7, no. 7, pp. 2466–2472, 2007.
- [172] A. R. Murray, E. Kisin, S. S. Leonard et al., "Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes," *Toxicology*, vol. 257, no. 3, pp. 161–171, 2009.
- [173] B. Chen, Y. Liu, W. M. Song, Y. Hayashi, X. C. Ding, and W. H. Li, "In vitro evaluation of cytotoxicity and oxidative stress induced by multiwalled carbon nanotubes in murine RAW 264.7 macrophages and human A549 Lung cells," *Biomedical and Environmental Sciences*, vol. 24, no. 6, pp. 593–601, 2011.
- [174] M. Pacurari, Y. Qian, W. Fu et al., "Cell permeability, migration, and reactive oxygen species induced by multiwalled carbon nanotubes in human microvascular endothelial cells," *Journal of Toxicology and Environmental Health A*, vol. 75, no. 2, pp. 112–128, 2012.